THE DIFFERENT COLOUR VARIETIES OF THE ASIAN AROWANA *SCLEROPAGES FORMOSUS* (OSTEOGLOSSIDAE) ARE DISTINCT SPECIES: MORPHOLOGIC AND GENETIC EVIDENCES

by

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ABSTRACT. - Description of three new species within *Scleropages formosus*. Phylogenetic analysis of partial mitochondrial sequence variation of the cytochrome b gene was undertaken on several colour varieties of *S. formosus* and revealed that each colour variant represented a distinct monophyletic entity. Because these results were supported by morphological data, it is concluded that *S. formosus* is composed of four distinct species, of which three are new to science. *Scleropages formosus* (Müller & Schlegel, 1844) is restricted to the green variety and is characterised by long maxillaries reaching far behind the eyes (upper jaw length: 106.2-109.2% HL), a low head depth (86.5-101.2% HL), a narrow head width (65.3-74.0% HL), a long pre-pectoral length (25.0-27.2% SL), a short pectoral-pelvic length (27.8-30.4% SL) a short pre-anal length (68.6-72.4% SL) and a short anal fin length (25.9-28.0% SL). *Scleropages macrocephalus* sp. nov. also known as the Silver variety in the aquarium trade (including those with yellow or grey tails) is characterised by long maxillaries reaching the posterior border of eyes (upper jaw length: 106.3-108.9% HL), a high head depth (106.3-116.0% HL), a broad head width (76.3-82.5% HL), a long pre-pectoral length (25.3-27.4% SL), a long pectoral-pelvic length (31.2-35.3% SL), a long pre-anal length (73.2-77.2% SL) and a short anal fin length (24.7-27.6% SL). *Scleropages aureus* sp. nov. known as the Red Tail Golden in the aquarium trade is distinguished by short maxillaries never reaching the posterior border of eyes (its length: 99.1-101.8% HL), a high head depth (104.8-110.0% HL), a narrow head width (68.6-73.8% HL), a relative intermediate pre-pectoral length (23.9-24.6% SL), a short pectoral-pelvic length (28.3-30.1% SL), a short pre-anal length (68.9-71.0% SL) and a long anal fin (28.7-29.6% SL). *Scleropages legendrei* sp. nov. which is known as the Super Red arowana in the aquarium trade is diagnosed by its very short maxillaries only reaching the middle of eyes (its length: 95.9-97.7% HL), a low head depth (90.1-102.3% HL), a narrow head width (65.9-73.9% HL), a short pre-pectoral length (22.2-23.4% SL), a short pectoral-pelvic length (28.6-30.6% SL), a short pre-anal length (68.3-72.1% SL) and a short anal fin (25.6-27.9% SL). The occurrence of first generation hybrids specimens was observed in some Indonesian farms mainly between *S. legendrei* and *S. macrocephalus*. These specimens are easily distinguished from their parental species by their intermediate morphological and molecular characters.

RÉSUMÉ. - Les différentes variétés de coloration chez l'arowana asiatique, *Scleropages formosus* (Osteoglossidae) sont des espèces distinctes : évidences génétiques et morphologiques.

L'analyse des séquences du gène du cytochrome b effectuée sur plusieurs variétés de coloration de l'arowana asiatique *Scleropages formosus* montre que ces variétés représentent des entités phylogénétiques distinctes. Une étude biométrique effectuée sur 36 spécimens confirme les résultats moléculaires et démontre l'existence de trois nouvelles espèces au sein de *S. formosus*. *Scleropages formosus* (Müller & Schlegel, 1844) qui correspond à la variété verte se caractérise par de longs maxillaires qui dépassent largement la bordure postérieure des yeux (106,2-109,2% HL), une tête étroite (65,3-74,0% HL) et de faible épaisseur (86,5-101,2% HL), une longue distance prépectorale (25,0-27,2% SL), une courte distance entre la base des nageoires pectorales et pelviennes (27,8-30,4% SL), une courte distance préanale (68,6-72,4% SL) et une courte nageoire anale (25,9-28,0% SL). *Scleropages macrocephalus* sp. nov. (variété argentée) se caractérise par la combinaison des caractères suivants: de longs maxillaires atteignant la bordure postérieure des yeux (106,3-108,9% HL), une tête large (76,3-82,5% HL) et haute (106,3-116,0% HL), une longue distance prépectorale (25,3-27,4% SL), une longue distance entre la base des nageoires pectorales et pelviennes (31,2-35,3% SL), une longue distance préanale (73,2-77,2% SL) et une courte nageoire anale (24,7-27,6% SL). *Scleropages aureus* sp. nov. (variété dorée) se distingue par de courts maxillaires qui n'atteignent pas la bordure postérieure des yeux (99,1-101,8% HL), une tête étroite (68,6-73,8% HL) et haute (104,8-110,0% HL), une distance prépectorale intermédiaire comparée aux autres espèces (23,9-24,6% SL), une courte distance entre la base des nageoires pectorales et pelviennes (28,3-30,1% SL), une courte distance préanale (68,9-71,0% SL) et une longue nageoire anale (28,7-29,6% SL). *Scleropages legendrei* sp. nov. (variété rouge) se différencie par de très courts maxillaires qui ne dépassent pas le milieu des orifices orbitaux (95,9-97,7% HL), une tête étroite (65,9-73,9% HL) et de faible épaisseur (90,1-102,3% HL), une courte distance prépectorale (22,2-23,4% SL), une courte distance entre la base des nageoires pectorales et pelviennes (28,6-30,6% SL), une courte distance préanale (68,3-72,1% SL) et une courte nageoire anale (25,6-27,9% SL). L'existence d'hybrides a également été mise en évidence entre *S. legendrei* et *S. macrocephalus*, ces spécimens étant facilement reconnaissables par leurs caractéristiques morphologiques et moléculaires intermédiaires.

Key words. - Osteoglossidae - *Scleropages formosus* - *S. macrocephalus* - *S. aureus* - *S. legendrei* - Asian arowana - Coloured varieties - Phylogeny - Taxonomy - New species.

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The order Osteoglossiformes represents one of the primary freshwater fish groups that are strictly intolerant of saltwater (Banarescu, 1990) and are considered as the living sister group of all other living teleosts (Hilton, 2003). Osteoglossiformes were divided into two suborders, *i.e.* Notopteroidei and Osteoglossoidei (Nelson, 1994). The former comprises Old World knifefishes (Notopteridae), elephantfishes (Mormyridae), mooneyes (Hiodontidae), and the aba (Gymnarchidae), while the latter includes arowanas (Osteoglossidae) and butterflyfish (Pantodontidae). The Osteoglossidae have a wide range of body shapes and lifestyles, but all of them share the common characteristics of at least partial air breathing ability and well-developped parental care, from mouth brooding to nest guarding (Greenwood and Wilson, 1998). The bony tongue family Osteoglossidae comprises four genera, *Osteoglossum* and *Arapaima* in South America, *Heterotis* in Africa, and *Scleropages* in Southeast Asia and the Australian region (Nelson, 1994; Greenwood and Wilson, 1998).

The genus *Scleropages* Günther, 1864 includes three species: the northern barramundi *Scleropages jardinii* (Saville-Kent, 1892) distributed in Northern Australia and Southern New Guinea, the spotted barramundi *Scleropages leichardti* Günther, 1864 confined to Eastern Australia and finally the Asian arowana *Scleropages formosus* (Müller & Schlegel, 1844) inhabiting Southeast Asia (south-eastern Thailand, south-western Kampuchea, southern Vietnam, Malay Peninsula, Sumatra, Borneo, Bangka). Despite an apparent close morphological similarity, Kumuzawa and Nishida (2000) suggest, based on molecular and geological evidence, that the Asian arowana vicariantly diverged from the Australasian arowanas in the eastern margin of Gondwanaland (early Cretaceous) and migrated into Eurasia on the Indian subcontinent or smaller continental blocks. According to Goh and Chua (1999) the spread of *S. formosus* in Southeast Asia is more recent, probably during the Pleistocene glacial ages when the Sunda shelf connected the major islands of the Indonesian archipelago with Indochina (Voris, 2000).

Over the past few decades, *Scleropages formosus* has acquired a special status in most Asian countries as a very popular but extremely expensive aquarium fish, which has led to overexploitation and its inclusion in the list of species threatened with extinction (Goh and Chua, 1999).

These authors note that there are basically 4 different natural occurring colour varieties for *S. formosus*. The "Green" is the most common variety and is widespread throughout Southeast Asia. The other varieties are confined to distinct and separate geographic areas: the "Super Reds" are only known from the upper part of the Kapuas River (Danau Sentarum Reserve, West Borneo, Indonesia), the "Cross Back Goldens" or "Blue Malayan" bonytongues are known from the Pahang State and Bukit Merah Lake (Malay Peninsula, Malaysia), the "Red Tail Goldens" originate from the area of Pekanbaru and Jambi (North Sumatra, Indonesia).

The "Silver Asian" Arowana with grey or yellow tail and included within the Greens by Goh and Chua (1999) is also a distinct variety following many Indonesian fish dealers and farmers. The "Grey Tail Silver" or "Pinoh Arowana" originates from the Schwaner Range separating West and Central Kalimantan (Borneo, Indonesia), while the "Yellow Tail Silver" occurs in Central and South Kalimantan (Borneo, Indonesia).

With a presumed resemblance to the "Chinese Ancient Dragon", many Chinese believe that the Super Red (Dragon Fish) symbolises luck, wealth, prosperity and strength. Due to its scarcity and its popularity, this variety has the highest commercial value and costs in Indonesia as much as US$ 200 per fingerling and between 1,000 and 2,000 US$ for a specimen of 30 cm length.

While studying samples of *Scleropages formosus*, some colour varieties appeared to have diagnostic morphological characters. A closer morphological examination coupled with genetic divergence estimations and ecological data revealed that most of these colour varieties are not conspecific. In the present paper, three species new to science are described and *S. formosus* is re-described.

MATERIALS AND METHODS

Material

The Asian arowana *Scleropages formosus* is a species protected by CITES (Goh and Chua, 1999). We studied 36 specimens representing all known colour varieties (except the Cross Back Golden) that died since 1998 after being caught by fishermen or kept and bred in tanks either by Indonesian governmental research institutions or by private fish farmers (see table I for colour variety designation and origin). Comparative material included three individuals of *S. formosus* from Cambodia and housed in the Muséum national d'Histoire naturelle (MNHN, France) and six wild specimens of *Scleropages jardinii* kindly given by the Indonesian Research Institute of Freshwater Fisheries (Tab. I). All specimens of Asian arowana and northern barramundi collected for the present study were deposited in the Museum Zoologicum Bogoriense, Cibinong (Indonesia) (MZB).

Molecular analysis

Mitochondrial analysis was performed in the Molecular Laboratory of the Indonesian Research Institute of Freshwater Fisheries (Pasar Minggu, Jakarta, Indonesia) and consisted in sequencing of anterior part of the cytochrome b (CytB)

Table I. - Colour variety designation, breeding source and geographic origins of material of *S. formosus sensu lato* and *S. jardinii* studied in genetic and morphology. ***: Specimen of the Super Red variety caught in wild and immediately released after the sampling of a small piece of the caudal fin for molecular analysis.

Species	Colour variety	Registration number	Origin
Scleropages formosus	Green	MZB 11888-11889	Nanga Pinoh market, wild origin (Melawi River, West Borneo, Indonesia)
	Green	MZB 11890-11891	Nanga Pinoh market, wild origin (Pinoh River, West Borneo, Indonesia)
	Green	MZB 11887	Wild specimen (Barito River, Central Borneo, Indonesia)
	Green	MZB 11892-11894	Wild specimens (Lalang River, Sumatra, Indonesia)
	Green	MZB 11895-11897	Wild specimens (Batanghari River, Sumatra, Indonesia)
	Green	MNHN 12415-12417	Wild specimens (Cambodia)
	Yellow Tail Silver	MZB 11899	RIFF strain, wild specimen (Barito River, Central Borneo, Indonesia)
	Yellow Tail Silver	MZB 11900-11902	Jakarta market, cultivated origin from private strain (Java, Indonesia)
	Grey Tail Silver	MZB 11898	Nanga Pinoh market, wild origin (Melawi River, West Borneo, Indonesia)
	Grey Tail Silver	MZB 11903-11905	Jakarta Market, cultivated origin from private strain (Java, Indonesia)
	Red Tail Golden	MZB 11906	Pekanbaru Market, wild origin (Siak River, Sumatra, Indonesia)
	Red Tail Golden	MZB 11907-11911	Jakarta Market, cultivated origin from private strain (Java, Indonesia)
	Super Red	MZB 11913	Semitau strain, cultivated origin, first generation from wild broodstock (West Borneo, Indonesia)
	Super Red	***	Wild specimen, (Meliau, Sentarum Lake, West Borneo, Indonesia)
	Super Red	MZB 11914-11915	Jakarta market, cultivated origin from private strain (Java, Indonesia)
	Super Red	MZB 11912	Private strain from Bogor, wild specimen (Java, Indonesia)
	Super Red	MZB 11916-11917	Private strain from Bogor, cultivated origin (Java, Indonesia)
Scleropages jardinii	northern barramundi	MZB 11881-11886	RIFF strain, wild specimens (Mereoke, Irian Jaya, Indonesia)

gene. Small pieces of muscle (100 mg) were used to isolate total genomic DNA using an hexa decyl methyl ammonium bromide extraction process (Doyle and Doyle, 1987).

Portion of the CytB gene was amplified with primers L15267 (5'-AATGACTTGAAGAACCACCGT-3') and H15891 (5'-GTTTGATCCCGTTTCGTGTA-3') (Briolay *et al*., 1998).

Mitochondrial DNA amplification was performed in 50 μl reaction volume containing 2 units of *Taq* polymerase (Promega), 1X reaction buffer (Promega), 1 μM of each primer, 0.18 mM of each dNTP, 1.8 mM $MgCl_2$ and 1 μl (50-100 ng) of total DNA. Amplification procedure consisted with a cycle of three steps (94°C for 2 min, 54°C for 30 s, 72°C for 40 s) repeated 30 times. Amplified mitochondrial fragments were electrophoresed on a 0.6% agarose gel and visualised using ethidium bromide staining. The remaining amplified product was purified using Glassmilk silica matrix of the Gene-Clean II kit from Bio 101, Inc. The purified mtDNA was used directly in dideoxy-termination sequencing reaction (Sanger *et al*., 1977) with fluorescent-labelled primers (Pharmacia) and the Thermo-Sequenase sequencing kit with 7-deaza-dGTP (Amersham).

Sequencing conditions consisted of an initial denaturation at 95°C for 4 min 30 s followed by 25 cycles at 95°C for 30 s and 60°C for 30 s. Products were run out on a denaturing 6% acrylamide gel (Biorad) and visualised on a Pharmacia automated sequencer. In order to check sequence accuracy and to correct any ambiguity bases, both strands

were sequenced using each one of the two initial PCR primers. Sequences of both strands were compared with each other and aligned to the complete mitochondrial sequence of *Osteoglossum bicirrhosum* (the silver Amazonian arowana; gb: AB 043025; Inoue *et al*., 2001 using the sequence editor ESEE (version 3.1 s; Cabot and Beckenbach, 1989).

Phylogenetic inference was based on the neighbour joining (Saitou and Nei, 1987) method (NEIGHBOR program in PHYLIP; Felsenstein, 1993) from Kimura's distances among species and on the Wagner parsimony method (DNAPARS program in PHYLIP; Felsenstein, 1993). The reliability of the topologies was assessed with bootstrap from 1000 replicates (SEQBOOT and CONSENSE programs in PHYLIP; Felsenstein, 1993). In order to root the algorithms, we sequenced specimens of *Osteoglossum bicirrhosum* (Silver Amazonian arowana), *Osteoglossum ferreirai* (Black Amazonian arowana) and *Scleropages jardinii* (northern barramundi from Irian Jaya, Indonesia) originating from Indonesian Strains and a specimen of *Heterotis niloticus* (African arowana) sampled in Ivory Coast. For the same purpose, we also obtained from the GENBANK database the complete cytochrome b sequences of *Scleropages leichardti* (the Australian spotted barramundi; gb: AB035237; Kumazawa and Nishida, 2000), of *S. jardinii* (gb: AB035236; Kumazawa and Nishida, 2000) and of *Arapaima gigas* (the Amazonian pirarucu; gb: AB035241; Kumazawa and Nishida, 2000).

Morphological analysis

On each specimen, the following 23 measurements were taken using dial callipers and data were recorded to 0.1 mm: standard length (SL), from the tip of the snout (premaxilla) to the centre base of the caudal fin; head length (HL), from the tip of the snout to the posterior margin of occiput; head width (HW), measured on a vertical axis along the posterior margin of the occiput; head depth (HD), measured along a line crossing perpendicularly the top of the head just above the eye, through the middle of the eye, to the base of the lower jaw; upper jaw length (UJL), from the tip of the snout to posterior margin of premaxilla; lower jaw length (LJL), from the tip of the chin to posterior margin of the mandible; anterior snout length (ASNL), from the tip of the snout to the posterior nostril; snout length (SNL), from the tip of the snout to the anterior margin of eye; eye diameter (ED), vertically from upper to lower border of orbital cavity; pre-pectoral length (PPEL), from the tip of the snout to the base of first pectoral fin ray; pre-pelvic length (PPL), from the tip of the snout to the base of first pelvic fin ray; pre-anal length (PAL), from the tip of the snout to the base of first anal fin ray; pre-dorsal length (PDL), from the tip of the snout to the base of first dorsal fin ray; pectoral-pelvic length (PEPL), from base of first pectoral and to base of first pelvic fin rays; pelvic-anal length (PPAL), from base of first pelvic fin ray to base of last anal fin ray; posterior body depth (PBD), maximal depth measured vertically in the anus region from the belly to the dorsum; anterior body depth (ABD), maximal value measured vertically from the belly to the dorsum in front of the pelvic fin base; pectoral fin length (PEFL), from base to tip of first pectoral fin ray; pelvic fin length (PFL), from base to tip of first pelvic fin ray; anal fin length (AFL), from base of first anal fin ray to base of last anal fin ray; dorsal fin length (DFL), from base of first dorsal fin ray to base of last dorsal fin ray; caudal peduncle depth (CPD), maximal value measured vertically from ventral base of the caudal peduncle to its dorsal margin; caudal peduncle length (CPL), from the base of last dorsal fin ray to the centre base of the caudal fin. Meristic counts include number of gill rakers on the first branchial arch, number of dorsal fin rays, number of anal fin rays, number of scales on the lateral line, the position of dorsal, caudal and anal fins and the coloration. All measurements except the standard length were expressed as ratios of a reference length. Measurements on the body as well as the head length were expressed as a percentage of the standard length. All other measurements on the head were expressed as a percentage of the head length.

A principal component analysis (PCA) was carried on the morphometric data using the CSS STATISTICA (StatSoft, Inc.) version 4.5 package. Measurements were log-transformed before the PCA was run on the covariance matrix (Bookstein *et al*., 1985). The first factor of this analysis is considered as the size factor and was not taken into account in order to minimise the effect of size differences between the samples. Missing data were casewise deleted.

RESULTS

Cytochrome b phylogeny

A total of 310 nucleotides encoding the anterior part of the cytochrome b gene were aligned for the 36 specimens of *Scleropages formosus sensu lato* (Tab. II). These nucleotide sequences were compared with the published sequence (Inoue *et al*., 2001; gb: AB 043025) of *Osteoglossum bicirrhosum*, in order to locate the replication origin of the studied gene and to check codon position. The first nucleotide of our alignment corresponds to the 94th nucleotidic site from the replication origin of the cytochrome b gene (32nd codon position). In the 310-bp sequences of *S. formosus sensu lato*, 10 (3.2%) sites were polymorphic, 9 of these (90%) were informative phylogenetically, and 8 (89%) of the phylogenetically informative sites occurred in the third position. When we take into account the different outgroup species (see sequences alignment on table II), the number of polymorphic sites rise to 109 (35%) with 73 (24%) phylogeneti-

Table II. - Sequence alignment (cytochrome b gene, 310 base pairs) among the different coloured varieties of *S. formosus sensu lato* and other osteoglossids as outgroup.

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A. gigas (gb: AB035241)                    AACTTCGGCT CCCTACTAGG ACTCTGCCTC ATCACCCAAA TTCTCACAGG TCTCTTCCTT GCAATACACT ACACTTCTGA
H. niloticus                               .....T.... ....C...T. T.....TT.A GC.GT..... .C..A..C.. ...A.....A ..T.....T. .T..C..A..
O. ferreirai (Indonesian strain)           .......T. .T........ T......T.A ..T.T..... .C........ C..A.....A ..C.....T. ....C..A..
O. bicirrhosum (gb: AB043025)              .......... .......... T......T.A ..T.T..... .C........ C..A...T.A ..C.....T. ....A..A..
O. bicirrhosum (Indonesian strain)         .......... .......... T......T.A ..T.T..... .C........ C..A...T.A ..C.....T. ....A..A..
S. jardinii (gb: AB035236)                 .......... ....C..G.. C........A G.GGT..... .C.....T.. C..A..TT.A ..T....... ....CG....
S. jardinii (wild, RIFF strain)            .......... ....C..G.. C........A G.GGT..... .C.....T.. C..A..TT.A ..T....... ....CG....
S. leichardti (gb: AB035237)               .......... .T..C..... C......T.A GGT.T..... .C..T..... C..A.....G ..C..G.... .T..C..C..
S. formosus (Super red)                    .......... ....T..... C........G GGAG.T.... ....A..... C........A ..C....... ....C.....
S. formosus (Red Tail Golden)              .......... ....T..... C........G GGGG.T.... ....A..... C........A ..C....... ....C.....
S. formosus (Grey Tail Silver)             ........A. ....T..... C........G GGGG..... ....A..... C........A ..C....... ....C.....
S. formosus (Yellow Tail Silver, wild)     ........A. ....T..... C........G GGAG..... ....A..... C........A ..C....... ....C.....
S. formosus (Yellow Tail Silver, cultivated) ........A. ....T..... C........G GGAG..... ....A..... C........A ..C....... ....C.....
S. formosus (Green, Sumatra)               .......... ....T..... C........G GGGG..... ....A..... C........A ..C....... ....C.....
S. formosus (Green, West Borneo, Melawi R.) .......... ....T..... C........G GGAG..... ....A..... C........A ..C....... ....C.....
S. formosus (Green, West Borneo, Pinoh R.) .......... ....T..... C........G GGGG..... ....A..... C........A ..C....... ....C.....
S. formosus (Green, Central Borneo)        .......... ....T..... C........G GGGG..... ....A..... C........A ..C....... ....C.....

A. gigas (gb: AB035241)                    CATTTCAACC GCCTTTTCTT CAGTAGCTCA CATCTGCCGA GACGTAAACT ATGGCTGATT AATCCGAAAT CTACACGCCA
H. niloticus                               ...C.....G ..T..C.... .T..C..C.. .....CA... .....C.... ....A...C. ......C.GC ..C..T..A.
O. ferreirai (Indonesian strain)           ...C.....T .....C.... .C.....C.. ...T..... .......... .C..A...C. ......C..C ..T.....A.
O. bicirrhosum (gb: AB043025)              ...C.....T .....C.... .......C.. .......... .......... .C..A...C. ......T..C ..T.....A.
O. bicirrhosum (Indonesian strain)         ...C.....T .....C.... .......C.. .......... .......... .C..A...C. ......T..C ..T.....A.
S. jardinii (gb: AB035236)                 .G.C.....T .....C..C. .T..G..C.. .......... .....T.... ........C. ......T..C ..C.....A.
S. jardinii (wild, RIFF strain)            .G.C.....T .....C..C. .T..G..C.. .......... .....T.... ........C. ......T..C ..C.....A.
S. leichardti (gb: AB035237)               ...C.....T .....C..C. .C.....C.. T........C A......... .C......C. T.....C..C ..T.....A.
S. formosus (Super red)                    ...C.....T .....C..C. .C.....C.. .......... .....C.... .C..A...C. C.....C..C ........A.
S. formosus (Red Tail Golden)              ...C.....T .....C..C. .C.....C.. T......... .....C.... .C..A...C. C.....C..C ........A.
S. formosus (Grey Tail Silver)             ...C.....T .....C..C. .C.....C.. T......... .....C.... .C..A..... C.....C..C ........A.
S. formosus (Yellow Tail Silver, wild)     ...C.....T .....C..C. .C.....C.. T......... .....C.... .C..A..... C.....C..C ........A.
S. formosus (Yellow Tail Silver, cultivated) ...C.....T .....C..C. .C.....C.. T......... .....C.... .C..A..... C.....C..C ........A.
S. formosus (Green, Sumatra)               ...C.....T .....C..C. .C.....C.. T......... .....C.... .C..A..... C.....C..C ........A.
S. formosus (Green, West Borneo, Melawi R.) ...C.....T .....C..C. .C.....C.. T......... .....C.... .C..A..... C.....C..C ........A.
S. formosus (Green, West Borneo, Pinoh R.) ...C.....T .....C..C. .C.....C.. T......... .....C.... .C..A..... C.....C..C ........A.
S. formosus (Green, Central Borneo)        ...C.....T .....C..C. .C.....C.. T......... .....C.... .C..A..... C.....C..C ........A.

A. gigas (gb: AB035241)                    ACGGTGCCTC CTTCTTTTTC ATTTGCATAT ACCTCCATGT AGCCCGAGGT TTATACTACG GCTCCTATGC CTATAAAGAA
H. niloticus                               ....C..... A.....C... ..C.....C. .......... .........C C.T.....T. ....T..CA. ..........
O. ferreirai (Indonesian strain)           .......... A.....C..T ..C.....C. ....G..C.. ...A.....C C.......T. ....A...CT G....T...G
O. bicirrhosum (gb: AB043025)              .......... A......... ..C.....C. ....A..C.. ...A.....C C.......T. ....G..CCT A.........
O. bicirrhosum (Indonesian strain)         .......... A......... ..C.....C. ....A..C.. ...A.....C C.......T. ....G..CCT A.........
S. jardinii (gb: AB035236)                 ....C..... AC.A..C... ..C.....C. ....A..C.. ...A.....C C.G....... .......CCT A..C..G...
S. jardinii (wild, RIFF strain)            ....C..... AC.A..C... ..C.....C. ....A..C.. ...A.....C C.......... .......CCT A..C..G...
S. leichardti (gb: AB035237)               .T..C..... A.....C... ........C. ..T.A..C.. ...A.....C C.......... .T.....CCT T.......G.
S. formosus (Super red)                    .......T.. A.....C... ..C.....C. ....T..C.. G..A.....C C.G....... .......CCT A..C......
S. formosus (Red Tail Golden)              .......T.. A.....C... ..C.....C. ....T..C.. G..A.....G C.G....... .G.....CCT A..C......
S. formosus (Grey Tail Silver)             ....C..T.. A.....C... ..C.....C. ....T..C.. G..A.....C C.G....... .......CCT A..C......
S. formosus (Yellow Tail Silver, wild)     ....C..T.. A.....C... ..C.....C. ....T..C.. G..A.....C C.G....... .......CCT A..C......
S. formosus (Yellow Tail Silver, cultivated) ....C..T.. A.....C... ..C.....C. ....T..C.. G..A.....C C.G....... .......CCT A..C......
S. formosus (Green, Sumatra)               ....C..T.. A.....C... ..C.....C. ....T..C.. G..A.....C C.G....... .......CCT A..C......
S. formosus (Green, West Borneo, Melawi R.) ....C..T.. A.....C... ..C.....C. ....T..C.. G..A.....C C.G....... .......CCT A..C......
S. formosus (Green, West Borneo, Pinoh R.) ....C..T.. A.....C... ..CA....C. ....T..C.. G..A.....C C.G....... .......CCT A..C......
S. formosus (Green, Central Borneo)        ....C..T.. A.....C... ..C.....C. ....T..C.. G..A.....C C.G....... .......CCT A..C......

A. gigas (gb: AB035241)                    ACATGAAACA  TTGGAGTAAT  TCTTCTCCTA  CTAGTAATAA  TAACTGCATT  CGTAGGATAC  GTTCTTCCCT
H. niloticus                               ..........  ........G.  A..AT.A..C  ..........  ....A..C..  T........T  ..C.......
O. ferreirai (Indonesian strain)           ..........  .C......G.  C..C.....C  .....T....  ....A..C..  T.........  ..C.......
O. bicirrhosum (gb: AB043025)              ..........  .C......G.  C........C  .....T....  ....A..C..  ..........  ........T.
O. bicirrhosum (Indonesian strain)         ..........  .C......G.  C........C  .....T....  ....A..C..  ..........  .......TGG
S. jardinii (gb: AB035236)                 ..C.......  .C..G.....  CT.A..A..C  ..C..C....  ....A..C..  T........T  ..C..C....
S. jardinii (wild, RIFF strain)            ..C.......  .C..G.....  CT.A..A..C  ..C..C....  ....A..C..  T........T  ..C..C....
S. leichardti (gb: AB035237)               ..C.......  .C..G.....  C..A..A..C  ..T..T....  ....A.....  T........T  ..C..C....
S. formosus (Super red)                    ..........  .C......G.  C..G..A..C  ..........  ....A..C..  ..........  ..C..C..A.
S. formosus (Grey Tail Silver)             ..........  .C......G.  C..G..A..C  ..........  ....A..C..  ..........  ..C..C..A.
S. formosus (Yellow Tail Silver, wild)     ..........  .C......G.  C..G..A..C  ..........  ....A..C..  ..........  ..C..C..A.
S. formosus (Yellow Tail Silver, cultivated) ..........  .C..G...G.  C..G..A..C  ..........  ....A..C..  ..........  ..C..C..A.
S. formosus (Red Tail Silver)              ..........  .C..G...G.  C..G..A..C  ..........  ....A..C..  ..........  ..C..C..A.
S. formosus (Green, Sumatra)               ..........  .C......G.  C..G..A..C  ..........  ....A..C..  ..........  ..C..C..A.
S. formosus (Green, West Borneo, Melawi R.) ..........  .C......G.  C..G..A..C  ..........  ....A..C..  ..........  ..C..C..A.
S. formosus (Green, West Borneo, Pinoh R.) ..........  .C......G.  C..G..A..C  ..........  ....A..C..  ..........  ..C..C..A.
S. formosus (Green, Central Borneo)        ..........  .C......G.  C..G..A..C  ..........  ....A..C..  ..........  ..C..C..A
```

cally informative sites.

The nearest neighbour dendrogram was inferred from the corrected Kimura's genetic distances pairwise matrix among taxa (Fig. 1).

The topology of the resulting tree confirms the monophyly of the genus *Scleropages* (bootstrap 63). Nevertheless the two Australasian species (*S. jardinii* and *S. leichardti*) are highly differentiated from each other [corrected nucleotidic divergence (ND): 14.9-15.3%] and also from the Asian species (*S. formosus sensu lato*) (ND: 14.3-16.0%). These important levels of genetic divergence calculated within the genus *Scleropages* are similar to those estimated between

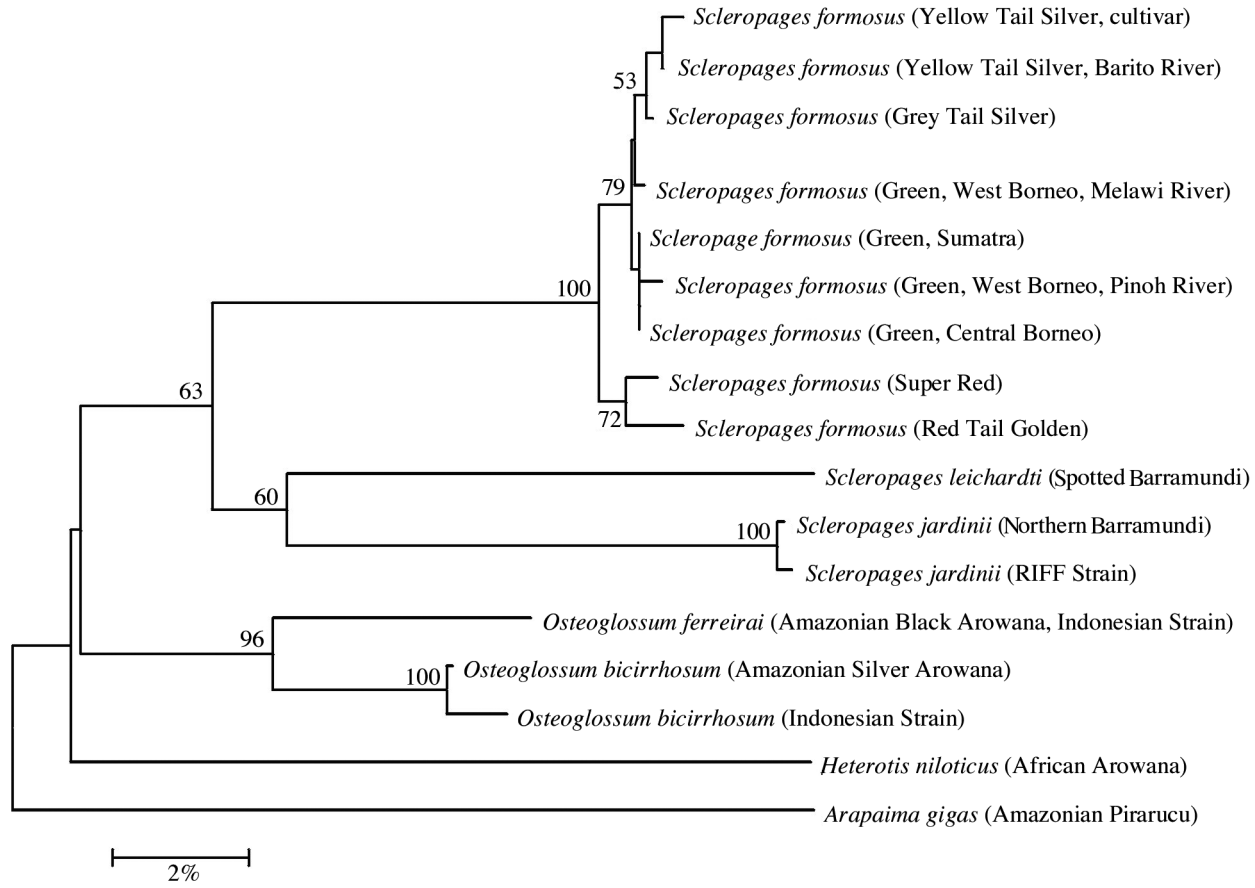

Figure 1. - A nearest neighbour dendrogram constructed from the partial cytochrome b sequences of the different coloured varieties of the Asian arowana and using other osteoglossids as outgroup. Numbers next to node show group occurrence found with the 1000 bootstrapped trees (in %).

this genus and the Amazonian genus *Osteoglossum* (ND: 13.1-18.6%). The classification of *S. formosus* and the two Australasian species within the same genus can therefore be doubted. The phylogenetic reconstruction performed from the analysis of parsimony shows the same results (not shown). Our results indeed confirm those of Kumazawa and Nishida (2000) which stated that the Asian arowana vicariantly diverged from the Australasian arowanas in the eastern margin of Gondwanaland.

The topology of the neighbour dendrogram (Fig. 1) also reveals that all the coloured varieties which compose *S. formosus sensu lato* represent a monophyletic entity (bootstrap 100). Within this group, the Super Reds and the Red Tail Goldens display a significant genetic relatedness (bootstrap 72) but differ by 1.3% of nucleotidic divergence. The Super Reds differ from the Greens by 1.3-2% and from the Silvers by 1.6-2.0%, while the Red Tail Goldens differ from the Greens by 1.6-2% and from the Silvers by 2.0-2.6%. The Silvers are more genetically related to the Green variety (bootstrap 79 and 0.3 < ND < 1.3%) but differ from the latter by the possession of one diagnostic substitution (A at the 9th position, bootstrap 53). The branching pattern within the group formed by the Silvers and the Green varieties suggests that the Silvers probably diverged recently from a green lineage.

Whatever their strain or wild origin, all analysed specimens of the Super Red variety possess the same cytochrome b sequence. This is also the case for the Red Tail Goldens. By contrast, three mitochondrial haplotypes were found within the green variety with two haplotypes found in West Borneo (each diagnostic for a particular river system), and one haplotype shared by populations from Central Borneo and various locations from Sumatra. Three distinct mitochondrial haplotypes were also found within the Silvers with one haplotype specific to all individuals of the Grey variety (wild or cultivated origins) and two haplotypes for the Yellow one. Nevertheless due to low quantities of genetic differentiation the bootstrap tests do not support the monophyly of the Yellow variety. The two Silver varieties are vicariantly distributed, the former confined to the upper part

of the Pinoh and the Melawi Rivers in the Schwaner Range (West Borneo, Indonesia), and the latter inhabiting the Barito River drainage (Central Borneo, Indonesia).

Based on these results, we strongly suspect that the Super Reds, the Red Tail Goldens, the Greens and the Silvers represent distinct biological entities, each characterised by diagnostic nucleotidic substitutions. These assumptions are enforced by the distinct ecological and geographic features which characterise most of these varieties. Following our field observations in Indonesia, the Super Reds are indeed confined to tannin stained blackwater streams with low pH values (< 5.5) in small forested lakes bordering the Sentarum Lake (upstream of Kapuas River, West Borneo, Indonesia). Interestingly, the Red Tail Goldens which display significant genetic relatedness with the Super Reds, inhabit the same type of habitat in the numerous lakes bordering the Siak River (Pekanbaru, Sumatra, Indonesia) or the Batanghari River (Berbak Reserve, Jambi, Sumatra, Indonesia). The Greens and the Silvers inhabit little streams of white or clear waters with higher pH values (> 6) and were never caught in sympatry with the formers varieties or in similar ecological habitats. Interestingly, the Silvers and the Greens which are phylogenetically distinct, occur sympatrically in many locations, suggesting their possible reproductive isolation.

Morphology

Following Kottelat *et al*. (1993), *Scleropages formosus* is easily distinguishable from *S. jardinii* and *S. leichardti* by a lower number of lateral line scales (21-24 *vs* 32-35). Our results based on a larger sample size (*S. formosus*, n = 40; and *S. jardinii*, n = 6) confirm this diagnostic meristic character (21-26 *vs* 34-36). The present work also reveals that *S. formosus* possesses longer pectoral fins (28.7-38.8 *vs* 24.6-28.4% SL), longer pelvic fins (12.2-18.6 *vs* 8.7-9.8% SL) and a longer anterior snout (22.5-29.3 *vs* 21.3-22.1% HL) than *S. jardinii*.

A PCA was carried out using 14 log-transformed measurements (excluding standard length, eye diameter, pelvic-anal length, pelvic and pectoral fin length, anterior and posterior body depth, depth and length of caudal peduncle) taken on 36 specimens of *S. formosus*. The plot of the second and the third factors of this PCA, explaining 0.12% of the total variation, is given in figure 2. The second factor is merely defined (in decreasing order of importance) by the dorsal fin length, the anterior snout length, the head depth, the head width and the pectoral-pelvic length. The third factor defined (in decreasing order of importance) by the anal fin length, the pre-pectoral length, the upper jaw length and the pre-pelvic length. The specimens of *S. formosus* belong to four distinct groups corresponding to distinct colour varieties. The Silvers are all situated on the negative sectors of the second axe and on the positive sector of the third axe, the Greens are mostly situated on the positive sectors of both axes, while the Super Reds and the Red Tail Golden are located on the negative sector of the third axe.

The plot of the upper jaw length with the pectoral-pelvic length (Fig. 3) or the pre-pectoral length with the pre-anal length (Fig. 4), enables the recognition of these distinct colour varieties:

- The Super Reds are characterised by a short upper jaw length (95.8-97.7% HL), a short pectoral-pelvic length (28.6-30.6% SL), a short pre-pectoral length (22.2-23.4% SL) and a short pre-anal length (68.3-72.1% SL).

- The Red Tail Goldens have an intermediate upper jaw length (99.1-101.8% HL), a short pectoral-pelvic length (28.3-30.1% SL), an intermediate pre-pectoral length (23.9-24.6% SL) and a short pre-anal length (68.9-71.0% SL).

- The Greens have a long upper jaw length (106.2-109.2% HL), a short pectoral-pelvic length (27.8-30.4% SL), a long pre-pectoral length (25.0-27.2% SL) and a short pre-anal length (68.6-72.4% SL).

- The Silvers with grey and yellow tails have a long upper jaw length (106.3-108.9% HL), a long pectoral-pelvic length (31.2-35.3% SL), a long pre-pectoral length (25.3-27.4% SL) and a long pre-anal length (73.2-77.2% SL).

The plotting of the anal fin length with the head depth (Fig. 5) also enables the recognition of the Red Tail Goldens from the Silvers and from the remaining Super Reds and Greens. The Red Tail Goldens are characterised by a long anal fin (28.7-29.6% SL) and a high head depth (104.8-110.0% HL) while the Silvers possess a short anal fin length (24.7-27.6% SL) and a high head depth (106.3-116.0% HL). The Super Reds and the Greens cannot be differentiated from each other but are easily distinguished from the Silvers and the Red Tail Goldens by a short anal fin (25.6-28.0% SL) and a short head depth (86.5-102.3% HL).

Finally, the head width also enables the recognition of the Silvers from all the other varieties, which are characterised by a broader head width (76.3-82.5 *vs* 65.3-74.0% HL). The varieties in tail coloration observed within the Silvers seem to represent a single morphologic entity because not a single morphological character enables their recognition.

Based on these genetic and morphologic evidence, we therefore conclude that the Super Red, the Red Tail Golden, the Green and the Silver varieties represent distinct species. Following Eschmeyer *et al*. (1998), no types of *S. formosus* are known, but the type locality is the Barito River (Central Borneo). The Greens and the Silvers are the two varieties which naturally inhabit the Barito river. We propose to restrict *S. formosus* to the Green variety following the description of *S. formosus* given by Weber and de Beaufort (1913): "dorsally dark olivaceous green, the sides and ventral surface silvery or golden green, sometimes with longitudinal rows of oblique dark patches, shining through the lat-

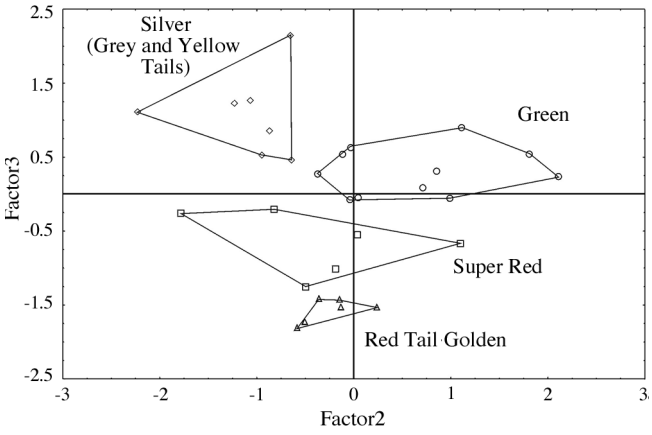

Figure 2. - Plot of the second and the third principal components of a PCA using 14 log-transformed measurements taken on 36 specimens of *Scleropages formosus*.

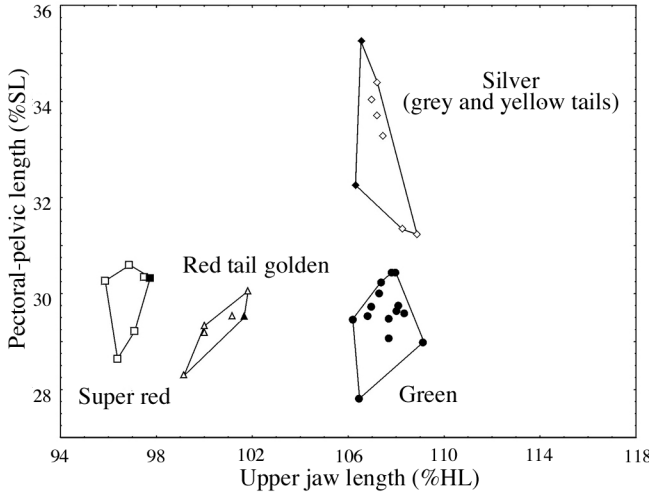

Figure 3. - Correlation between the upper jaw length (in% head length) and the pectoral-pelvic length (in% standard length) in the different coloured varieties of the Asian arowana (*S. formosus*). Black symbols refer to specimens from wild and blank symbols refer to cultivated specimens.

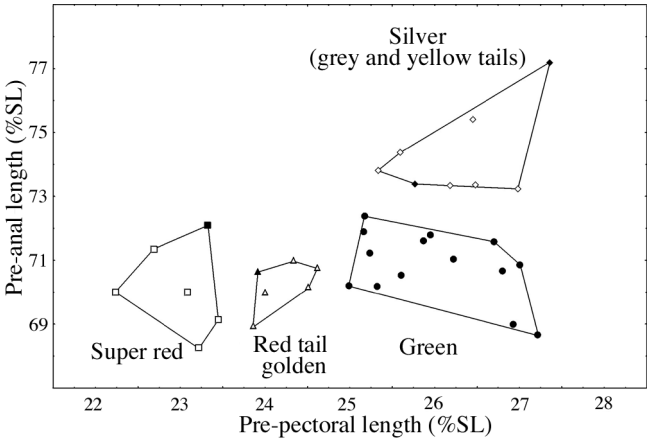

Figure 4. - Correlation between the pre-pectoral length (in% standard length) and the pre-anal length (in% standard length) in the different coloured varieties of the Asian arowana (*S. formosus*). Black symbols refer to specimens from wild and blank symbols refer to cultivated specimens.

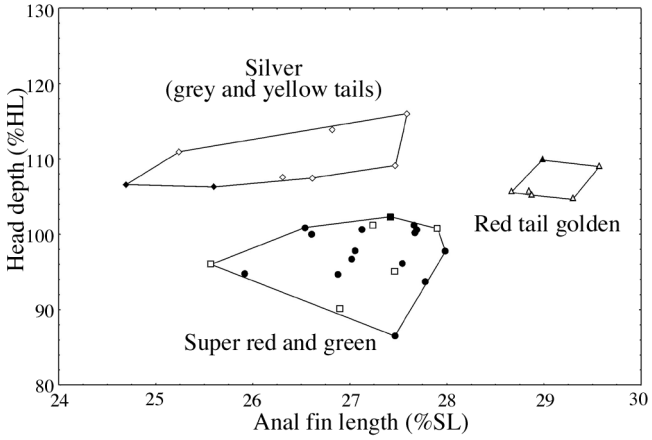

Figure 5. - Correlation between the anal fin length (in% standard length) and the head depth (in% head length) in the different coloured varieties of the Asian arowana (*S. formosus*). Black symbols refer to specimens from wild and blank symbols refer to cultivated specimens.

eral scales, fin membranes bluish, the rays reddish brown". The Silvers at the type locality are characterised by a silvery ventral surface, with no longitudinal rows of oblique patches but with the possession of a dark ring on most of the lateral scales, yellowish fin membranes with greyish dark rays.

SCLEROPAGES FORMOSUS (MÜLLER & SCHLEGEL, 1844)

(Figs 6, 7)

Material examined

Neotype (designated in this paper). - MZB 11887, 169 mm standard length, immature specimen collected in the Barito River near Buntok, Central Kalimantan (Borneo, Indonesia), coll. Sudarto, 1995.

Other specimens examined. - MNHN 12415-17, 3 specimens, 306-317 mm SL, Cambodia; MZB 11888-11889, 2 spms, 218-262 mm SL, Nanga Pinoh market, wild specimens from Melawi River, West Kalimantan (Borneo, Indonesia), coll. Sudarto, March 2000; MZB 11890-11891, 2 spms, 150-212 mm SL, Nanga Pinoh market, wild specimens from Pinoh River, West Kalimantan (Borneo, Indonesia), coll. Sudarto, March 2000; MZB 11892-11894, 3 spms, respectively 187, 328 and 390 mm SL, Bayung Lincir, Lalang River, Sumatra (Indonesia), coll. Sudarto, June 2000; MZB 11895-11897, 3 spms, respectively 285, 207 and 218 mm SL, Muaratebo, Batanghari River, Sumatra (Indonesia), coll. Sudarto, June 2000.

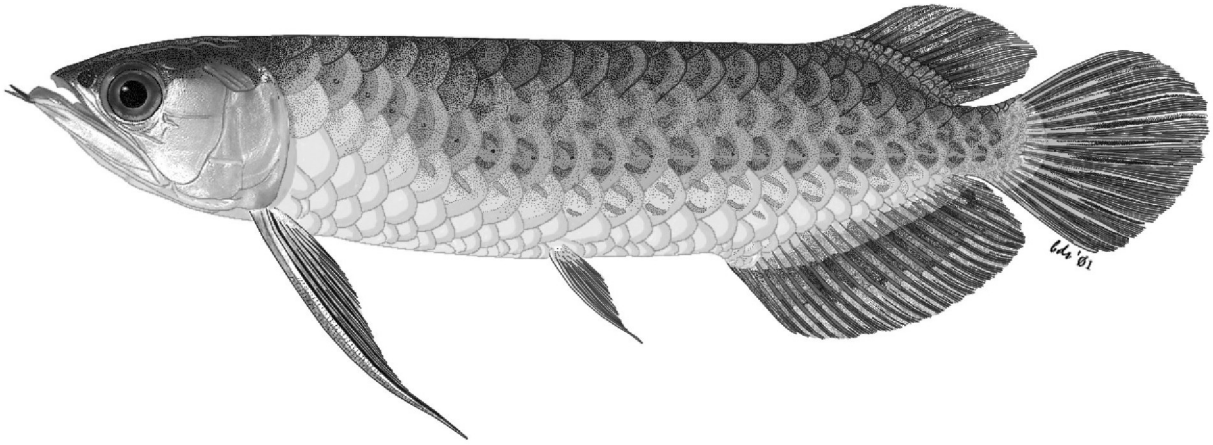

Figure 6. - *Scleropages formosus*: lateral view of a specimen of 262 mm SL (MZB 11889).

Diagnosis

depth about equal to its length, its width less than 3/4 its

Figure 7. - The pattern of coloration of *Scleropages formosus*. Lateral view of a specimen of 262 mm SL (Green variety, MZB 11889).

Scleropages formosus is distinguished from all other *Scleropages* species by the combination of the following characters: 23-26 scales on the lateral line, 25-28 anal fin rays, 17-19 dorsal fin rays, long maxillary reaching far behind eye (upper jaw length: 106.2-109.2% HL), a low head depth (86.5-101.2% HL), a narrow head width (65.3-74.0% HL), a long pre-pectoral length (25.0-27.2% SL), a short pectoral-pelvic length (27.8-30.4% SL) a short pre-anal length (68.6-72.4% SL) and a short anal fin length (25.9-28.0% SL).

Description

Based on the neotype and 13 specimens examined. Measurements are given in table III.

The body is elongate and slender, ventrally compressed: anterior and posterior body depths are about one quarter of standard length (Fig. 6). The head is long and slender, its length. Gape of mouth is oblique and maxillary long, reaching far behind the posterior border of eye. The snout is pointed and the chin is prominent with two stout fleshy barbels. The pectoral fins are long (about 3 times in standard length) and reach the base of pelvic fins which are about two times shorter. There are always 7 pectoral fin rays and 5 pelvic fin rays. The anal fin is relatively short (less than 28% of standard length) with 25-28 anal fin rays (25 in the neotype). The dorsal fin is short, between 1/2 and 3/4 of the anal fin. There are 17-19 dorsal fin rays (17 in the neotype). The number of gill rakers on the first branchial arch varies between 14 and 19. The number of scales on the lateral line varies between 23 and 26 (26 for the neotype).

Coloration (Fig. 7)

Dorsally dark olivaceous green, the sides silvery or golden green. Longitudinal rows of oblique dark greenish or

Table III. - Measurements for the neotype and 13 other specimens examined of *Scleropages formosus*. For abbreviations see Materials and Methods.

	Neotype	Other specimens examined				
SL (mm)	169	150 - 390				
In % standard length		n	min	max	mean	SD
HL	16.8	13	15.1	17.3	16.0	0.6
PPEL	27.2	13	25.0	27.0	25.9	0.7
PPL	54.3	13	53.4	55.7	54.5	0.7
PAL	68.6	13	69.0	72.4	71.0	0.9
PDL	77.1	13	76.4	79.3	77.7	1.0
PEPL	29.0	13	27.8	30.4	29.6	0.7
PPAL	42.1	13	41.9	44.5	43.5	0.8
PBD	24.7	13	22.7	25.0	24.0	0.7
ABD	24.6	13	23.7	25.6	24.6	0.7
PEFL	34.6	11	29.8	38.8	33.5	2.6
PFL	15.7	11	14.0	18.6	15.8	1.4
AFL	27.7	13	25.9	28.0	27.2	0.6
DFL	16.0	13	15.6	18.7	17.2	1.1
CPD	7.7	12	7.1	8.2	7.6	0.3
CPL	7.2	12	6.9	9.0	7.7	0.6
In % head length						
HW	65.5	10	65.3	74.0	70.2	2.4
HD	100.4	13	86.5	101.2	97.0	4.1
UJL	109.2	13	106.2	108.3	107.4	0.7
LJL	119.7	13	112.7	118.7	116.1	1.6
ASNL	26.1	13	25.0	28.8	26.4	1.4
SNL	42.3	13	38.5	41.1	40.0	0.8
ED	43.0	13	32.2	44.4	38.4	3.6

dark bluish patches are observed through the lateral scales. These patterns of coloration may vary with stress condition of the fish. The ventral surface is silvery or whitish. Fin membranes are bluish purple for specimens from Sumatra and are greenish purple for other locations. The rays are reddish brown. On mature specimens, the postocular part of head and the upper part of eye are bright emerald.

Habitat and ecology

Scleropages formosus inhabits slow flowing streams of more or less turbid white waters (pH > 6) generally bordering the main river course. This species is a mouth brooder and the males carry the eggs and larvae in the mouth until the yolk sac has been completely absorbed. Eggs are big averaging 1 cm in diameter. Larvae are between 6 and 7 cm length at birth. They mature at 3 years of age and rarely reach more than 60 cm of total length. Following the farmers, they have a fecundity comprised between 50 and 150 eggs. Juveniles feed on insects or small fish at the water surface and adults prey on fishes, frogs and big insects.

Distribution

Scleropages formosus is found in freshwater bodies of Southeast Asia including southern Thailand, Cambodia, southern Vietnam, Malay Peninsula and most river drainages of Borneo and Sumatra (Roberts, 1989) (Fig. 14).

Etymology and common names

Formosus: Given by Müller and Schlegel (1839-44) referring to its occurrence in Formosa. The species is commonly called the Asian bonytongue or Green Arowana.

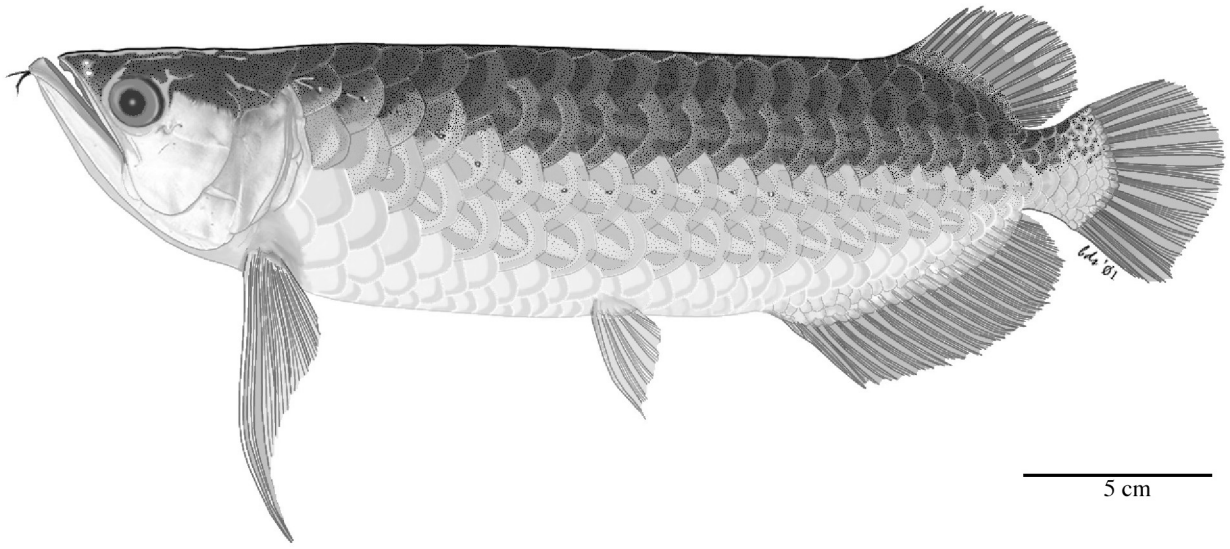

Figure 8. - *Scleropages macrocephalus* sp. nov.: lateral view of the holotype (MZB 11898).

SCLEROPAGES MACROCEPHALUS SP. NOV.
(Figs 8, 9 A-B)

Osteoglossum formosum Müller & Schlegel, 1844: 1 (type locality Barito River) (in part).

Scleropages formosus Weber & de Beaufort, 1913: 13 (in part).

Material examined

Holotype. - MZB 11898, 293 mm standard length, immature specimen, Nanga Pinoh market, wild specimens from Melawi River, West Kalimantan (Borneo, Indonesia), coll. Sudarto, March 2000.

Paratypes. - MZB 11899, 390 mm SL, a wild specimen from a strain maintained by Research Institute for Freshwater Fisheries in Bogor and originating from the Barito River, Central Kalimantan (Borneo, Indonesia); MZB 11900-11902, three specimens, respectively 304, 313, 325 mm SL, Barito Market (Jakarta), cultured specimens from private strains and descendants of a wild broodstock from the Barito River (Central Borneo, Indonesia), coll. Sudarto, September 2000; MZB 11903-11905, three specimens, respectively 431, 311, 330 mm SL, Barito Market (Jakarta), cultured specimens from private strains and descendants of a wild broodstock from the Pinoh River (West Borneo, Indonesia), coll. Sudarto, September 2000.

Diagnosis

Scleropages macrocephalus is distinguished from all other *Scleropages* species by the combination of the following characters: 21-26 scales on the lateral line, 24-27 anal fin rays, 17-19 dorsal fin rays, long maxillary reaching the posterior border of eye (upper jaw length: 106.3-108.9% HL), a high head depth (106.3-116.0% HL), a large head width (76.3-82.5% HL), a long pre-pectoral length (25.3-27.4% SL), a long pectoral-pelvic length (31.2-35.3% SL), a long pre-anal length (73.2-77.2% SL) and a short anal fin length (24.7-27.6% SL).

Description

Based on the holotype (Fig. 8) and 7 paratypes examined. Measurements are given in table IV.

The body is broad: anterior and posterior body depth about three to four times in standard length. The head is relatively robust, its depth always larger than its length, its width more than 3/4 its length. Gape of mouth is oblique and maxillary long, reaching the posterior border of eye. The snout is pointed and aligned with the dorsal outline, the chin is prominent and robust with two stout fleshy barbels. The pectoral fins are long (always more than 3 times in standard length) but generally do not reach the base of pelvic fins which are about two times shorter. There are always 7 pectoral fin rays and 5 pelvic fin rays. The anal fin is relatively short (less than 28% of standard length) with 24-27 anal fin rays (24 in the holotype). The dorsal fin is short, between 1/2 and 3/4 of the anal fin. There are 17-19 dorsal fin rays (18 in the holotype). The number of gill rakers on the first branchial arch is 18 for the holotype. The number of scales on the lateral line varies between 21 and 26 (23 in the holotype).

Table IV. - Measurements for the holotype and 7 paratypes examined of *Scleropages macrocephalus* sp. nov. For abbreviations see Materials and Methods.

	Holotype	Paratypes			
SL (mm)	293	304 - 431			
In % standard length		n	min	max	mean	SD
HL	16.2	7	15.1	16.4	15.8	0.5
PPEL	25.8	7	25.3	27.4	26.3	0.7
PPL	56.9	7	55.5	60.3	57.4	1.8
PAL	73.4	7	73.2	77.2	74.4	1.5
PDL	79.5	7	76.4	80.1	78.6	1.4
PEPL	32.3	7	31.2	35.3	33.3	1.5
PPAL	41.0	7	42.0	46.1	43.2	1.5
PBD	26.0	7	25.4	27.5	26.4	0.8
ABD	26.7	7	27.2	28.7	28.0	0.6
PEFL	35.1	6	31.4	35.8	33.3	1.8
PFL	13.7	7	13.5	16.5	15.2	1.0
AFL	25.6	7	24.7	27.6	26.4	1.1
DFL	16.8	7	14.8	17.1	16.4	0.8
CPD	8.0	7	7.7	8.5	8.1	0.3
CPL	6.7	7	6.6	7.7	7.0	0.4
In % head length						
HW	78.9	7	76.3	82.5	78.8	2.3
HD	106.3	7	106.6	116.0	110.2	3.6
UJL	106.3	7	106.6	108.9	107.5	0.8
LJL	116.6	7	114.3	120.0	116.1	2.0
ASNL	24.4	7	22.6	26.6	24.8	1.7
SNL	40.6	7	27.2	43.8	38.8	5.4
ED	38.1	7	35.5	41.7	37.8	2.4

Coloration (Fig. 9 A-B)

Dorsally dark grey, the sides silver. Circular dark greyish or olivaceous ring patches are observed on most of the lateral scales. The fin membranes are yellowish with rays greyish dark on specimens from the Barito River (Central Borneo, Indonesia, Fig. 9A) and are dark greyish on specimens from the West Borneo (Fig. 9B). The ventral surface is silvery or whitish.

Habitat and ecology

Scleropages macrocephalus inhabits small streams of more or less turbid white waters (pH > 6) and is also observed in big rivers. In West Borneo, the species can also occur in fast flowing waters. This species is also a mouth brooder. Eggs are big, between 1.38 and 1.58 cm in diame-

Figure 9. - The patterns of coloration of *Scleropages macrocephalus* sp. nov. **A**: Lateral view of a paratype of 304 mm SL (Yellow Tail Silver; MZB 11900); **B**: Lateral view of the holotype (Grey Tail Silver; MZB 11898).

ter. Larvae are between 6 and 7 cm length at birth. Following the farmers, this species matures at age 3, has a low fecundity (less than 50 eggs) and seems to have slow growth rate in ponds where they rarely reach more than 40 cm of total length. A mature female of 313 mm SL (MZB 11901) and 552 g was found with 33 eggs.

Distribution

Scleropages macrocephalus is found in freshwater bodies of Central (Barito River) and West Borneo (Melawi and Pinoh Rivers, two tributaries of the Kapuas River) (Fig. 14).

Etymology and common names

Macrocephalus: Referring to its robust head. The species is commonly known as Silver Indonesian Arowana, Pinoh Arowana for the grey-tailed variety, and Kuning Banjar for the yellow-tailed variety.

SCLEROPAGES AUREUS SP. NOV.

(Figs 10, 11 A-B)

Material examined

Holotype. - MZB 11906, 276 mm standard length, immature specimen, Pekanbaru market, wild specimen from Siak River, Sumatra (Indonesia), coll. Sudarto, May 2000.

Paratypes. - MZB 11907-11911, five specimens, respectively 350, 285, 260, 300 and 355 mm SL, Barito Market (Jakarta), offspring from private strains located in Pekanbaru (Sumatra, Indonesia) and descendants of a wild broodstock from the Siak River (Sumatra, Indonesia), coll. Sudarto, September 2000.

Diagnosis

Scleropages aureus is distinguished from all other *Scleropages* species by the combination of the following characters: 24-26 scales on the lateral line, 26-28 anal fin

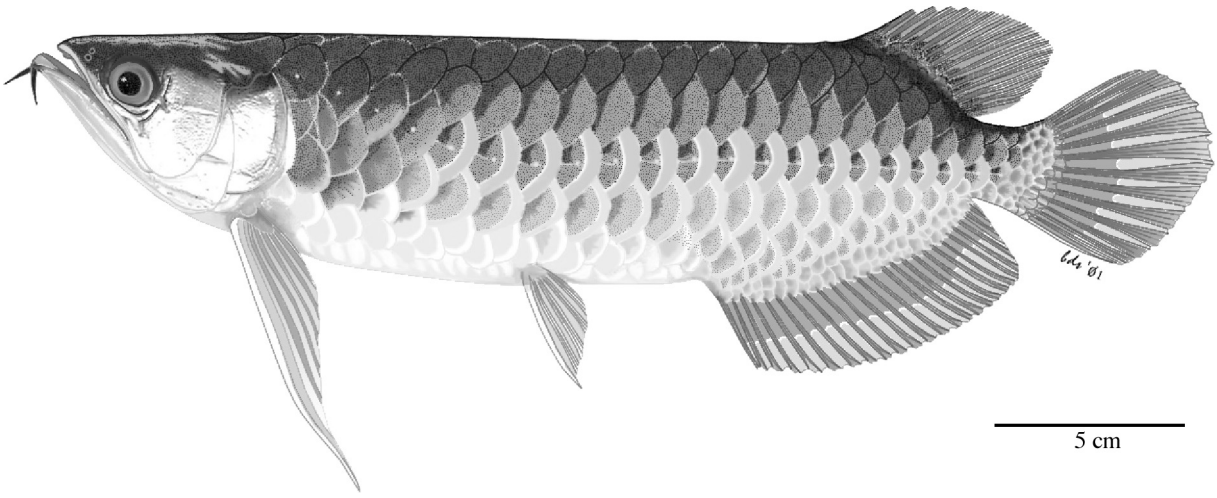

Figure 10. - *Scleropages aureus* sp. nov.: Lateral view of the holotype (MZB 11906).

Figure 11. - The patterns of coloration of *Scleropages aureus* sp. nov. **A**: Lateral view of the holotype (Red Tail Golden, immature specimen, MZB 11906); **B**: Lateral view of a paratype of 355 mm SL (Red Tail Golden, mature fish, MZB 11911).

rays, 15-20 dorsal fin rays, short upper jaw never reaching the posterior border of eye (its length: 99.1-101.8% HL), a high head depth (104.8-110.0% HL), a short head width (68.6-73.8% HL), a relative intermediate pre-pectoral length (23.9-24.6% SL), a short pectoral-pelvic length (28.3-30.1% SL), a short pre-anal length (68.9-71.0% SL) and a long anal fin (28.7-29.6% SL).

Description

Based on the holotype (Fig. 10) and 5 paratypes examined. Measurements are given in table V.

The body is broad: anterior and posterior body depth about three to four times in standard length. Head width less than 3/4 its length, but its depth always larger than its length. Gape of mouth is oblique and the upper jaw is short never reaching the posterior border of eyes. The snout is pointed and hooked, the chin is prominent and robust with two stout fleshy barbels. The pectoral fins are long (always more than 3 times in standard length) and reach the base of pelvic fins which are about two times shorter. There are always 7 pectoral fin rays and 5 pelvic fin rays. The anal fin is relatively long (more than 28% of standard length) with 26-28 anal fin rays (27 in the holotype). The dorsal fin is short, about the half of the anal fin. There are 15-20 dorsal fin rays (15 in the holotype). The number of gill rakers on the first branchial arch varies between 14 and 20 (14 in the holotype). The number of scales on the lateral line varies between 24 and 26 (24 in the holotype).

Table V. - Measurements for the holotype and 5 paratypes examined of *Scleropages aureus* sp. nov. For abbreviations see Materials and Methods.

	Holotype	Paratypes				
SL (mm)	293	304 - 431				
In % standard length		n	min	max	mean	SD
HL	16.2	7	15.1	16.4	15.8	0.5
PPEL	25.8	7	25.3	27.4	26.3	0.7
PPL	56.9	7	55.5	60.3	57.4	1.8
PAL	73.4	7	73.2	77.2	74.4	1.5
PDL	79.5	7	76.4	80.1	78.6	1.4
PEPL	32.3	7	31.2	35.3	33.3	1.5
PPAL	41.0	7	42.0	46.1	43.2	1.5
PBD	26.0	7	25.4	27.5	26.4	0.8
ABD	26.7	7	27.2	28.7	28.0	0.6
PEFL	35.1	6	31.4	35.8	33.3	1.8
PFL	13.7	7	13.5	16.5	15.2	1.0
AFL	25.6	7	24.7	27.6	26.4	1.1
DFL	16.8	7	14.8	17.1	16.4	0.8
CPD	8.0	7	7.7	8.5	8.1	0.3
CPL	6.7	7	6.6	7.7	7.0	0.4
In % head length						
HW	78.9	7	76.3	82.5	78.8	2.3
HD	106.3	7	106.6	116.0	110.2	3.6
UJL	106.3	7	106.6	108.9	107.5	0.8
LJL	116.6	7	114.3	120.0	116.1	2.0
ASNL	24.4	7	22.6	26.6	24.8	1.7
SNL	40.6	7	27.2	43.8	38.8	5.4
ED	38.1	7	35.5	41.7	37.8	2.4

Coloration (Fig. 11 A-B)

The dorsal region of this species is dark in colour including the dorsal fin and the third upper part of the dorsal fin. The other part of the dorsal fin and the anal fin are usually reddish in colour or light brown. On juveniles, the operculum, the scales on the lateral flanks, the ventral surface, the pectoral and pelvic fin membranes are silver with discrete golden reflection (Fig. 11A). The colour of these parts of the body becomes bright golden with age (Fig. 11B).

Distribution

Scleropages aureus is confined to tannin stained black water streams with low pH values (< 5.5) in small forested lakes bordering the Siak River in the area of Pekanbaru (Sumatra, Indonesia) or along the Batanghari River in the Berbak Reserve (Jambi, Sumatra, Indonesia) (Fig. 14).

Etymology and common names

Aureus: Referring to its gold coloration. The species is commonly known as the Red Tail Golden Arowana or Indonesian Golden.

SCLEROPAGES LEGENDREI SP. NOV.

(Figs 12, 13 A-C)

Material examined

Holotype. - MZB 11912, 620 mm standard length, a male from a private strain cultured in Bogor (West Java, Indonesia), originating from the area of the Sentarum Lake (West Borneo, Indonesia), coll. Sudarto, August 2000.

Paratypes. - MZB 11913, one specimen, 115 mm SL, offspring from a farm located at Semitau (Sentarum Lake, West Borneo, Indonesia), first generation from a wild broodstock, coll. Sudarto, March 2000; MZB 11914-11915, 2 specimens, 380-335 mm SL, Barito Market (Jakarta), offspring from private strains located in Pontianak (West Borneo, Indonesia); MZB 11916-11917, 2 spms, 290-405 mm SL, offspring from a private strain located in Bogor (West Java, Indonesia).

Diagnosis

Scleropages legendrei is distinguished from all other *Scleropages* species by the combination of the following characters: 23-25 scales on the lateral line, 25-27 anal fin rays, 17-19 dorsal fin rays, very short upper jaw reaching the middle of eye (its length: 95.9-97.7% HL), a short head

depth (90.1-102.3% HL), a short head width (65.9-73.9% HL), a short pre-pectoral length (22.2-23.4% SL), a short pectoral-pelvic length (28.6-30.6% SL), a short pre-anal length (68.3-72.1% SL) and a short anal fin (25.6-27.9% SL).

Description

Based on the holotype and 5 paratypes examined. Measurements are given in table VI.

The body is elongated and slender in immature specimens but becomes broader with age: anterior and posterior body depth are about four times in standard length in immature specimens and rise to three times in standard length on mature fish. The head is pointed (spoon head), long and slender, its width less than 3/4 its length, its depth about equal to its length (Fig. 12). Gape of mouth is very oblique; maxillary short, reaching approximately the middle of eye. The snout is short and pointed and the chin is prominent with two stout and long fleshy barbels (length always up to eye diameter in wild specimen). The pectoral fins are about 3 to 3.5 times in standard length and sometimes do not reach the base of pelvic fins which are short (about 2.5 times in pectoral fin length). There are always 7 pectoral fin rays and 5 pelvic fin rays. The anal fin is short (less than 28% of standard length) with 25-27 anal fin rays (25 in the holotype). The dorsal fin is short, about 1/2 to 3/4 of the anal fin. There are 17-19 dorsal fin rays (18 in the holotype). The number of gill rakers on the first branchial arch varies between 15 and 20 (15 in the holotype). The number of scales on the lateral line varies between 23 and 25 (24 in the holotype).

Table VI. - Measurements for the holotype and 5 paratypes examined of *Scleropages legendrei* sp. nov. For abbreviations see Materials and Methods.

	Holotype	Paratypes			
SL (mm)	620	115 - 405			
In % standard length		n	min	max	mean	SD
HL	14.2	5	15.2	17.8	16.1	1.1
PPEL	23.3	5	22.2	23.4	22.9	0.5
PPL	54.5	5	50.6	54.3	52.6	1.3
PAL	72.1	5	68.3	71.3	69.7	1.1
PDL	79.2	5	76.9	80.5	79.1	1.5
PEPL	30.3	5	28.6	30.6	29.8	0.8
PPAL	45.6	5	43.4	45.7	44.5	0.9
PBD	27.9	5	23.0	26.5	24.7	1.3
ABD	29.3	5	24.4	27.5	25.6	1.2
PEFL	31.5	5	28.7	37.0	33.2	3.6
PFL	15.0	5	12.2	18.3	15.0	2.5
AFL	27.4	5	25.6	27.9	27.0	0.9
DFL	17.4	5	14.8	16.7	15.5	0.7
CPD	7.2	5	7.4	8.1	7.7	0.2
CPL	7.5	5	7.0	7.6	7.3	0.2
In % head length						
HW	73.9	5	65.9	73.5	69.7	3.5
HD	102.3	5	90.1	101.2	96.7	4.6
UJL	97.7	5	95.9	97.5	96.7	0.6
LJL	110.5	5	104.9	113.5	109.8	3.6
ASNL	26.1	5	22.5	25.2	23.9	1.1
SNL	41.3	5	37.5	41.5	39.4	1.6
ED	30.1	5	34.3	42.9	37.7	3.4

Coloration (Fig. 13 A-C)

The dorsal region of this species is dark brown in colour. The lips are reddish. The lateral sides of the body, the operculum and the fins varies from goldish red (Fig. 13A) to deep red (Fig. 13B). On immature specimens the red color-

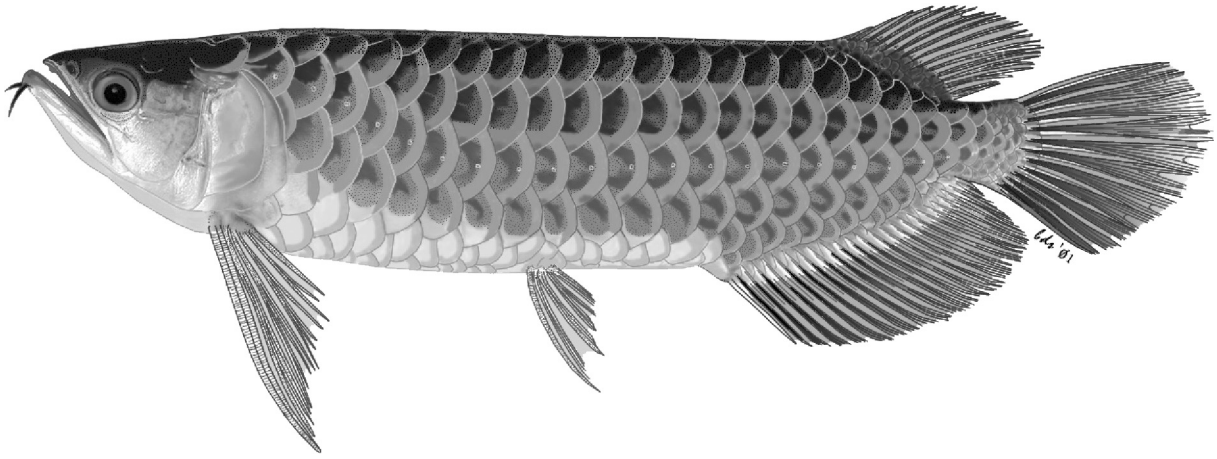

Figure 12. - *Scleropages legendrei* sp. nov.: Lateral view of a paratype of 380 mm SL (MZB 11914).

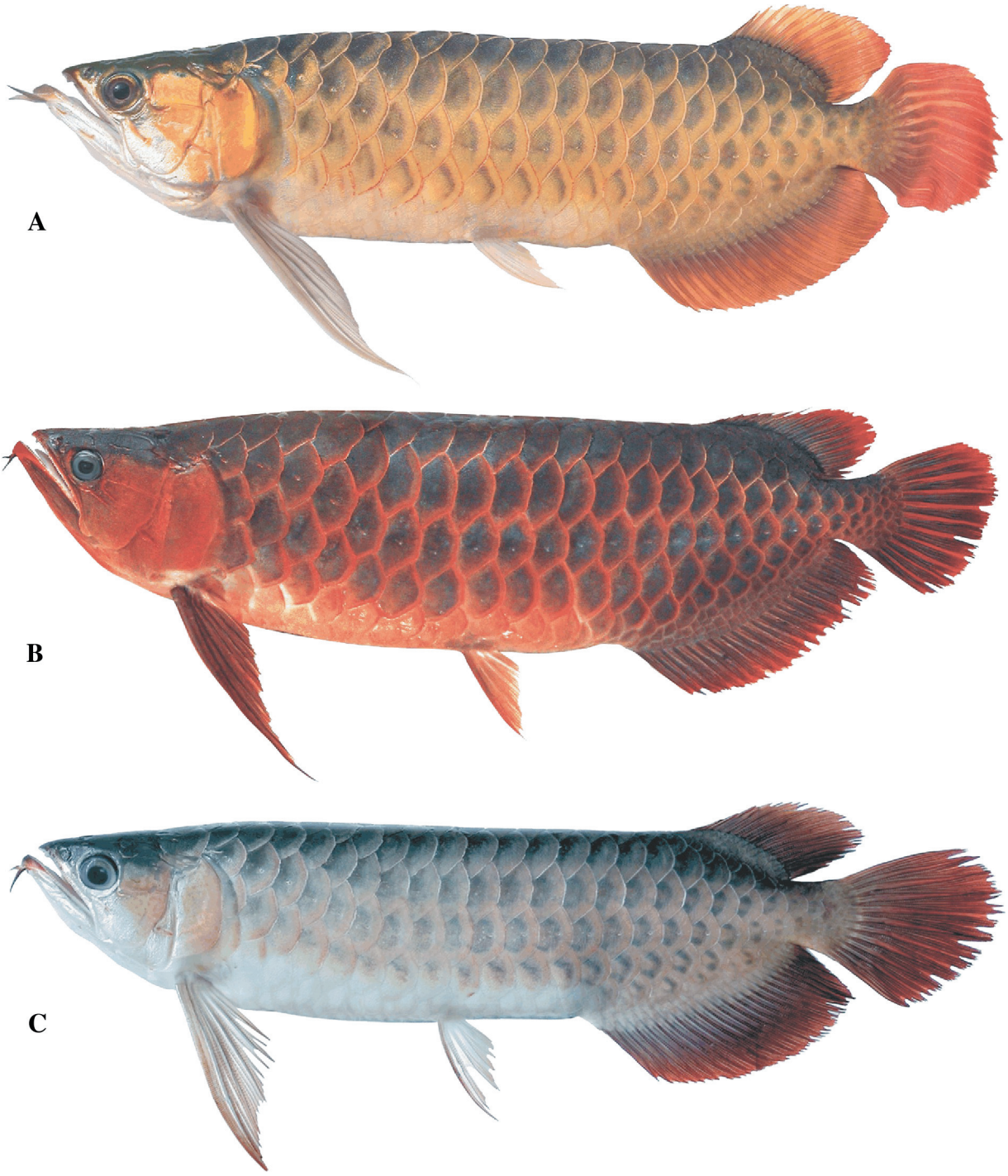

Figure 13. - The patterns of coloration of *Scleropages legendrei* sp. nov. **A**: Lateral view of a paratype of 405 mm SL (Super Red, mature male, MZB 11917); **B**: Lateral view of the holotype (Super Red, mature male, MZB 11912); **C**: Lateral view of a paratype of 380 mm SL (Super Red, immature fish, MZB 11914).

ation is less intense and the ventral surface is whitish (Fig. 13C). On juveniles, more darker is the top of the body, more deeper will be the operculum and scales coloration.

Distribution

Scleropages legendrei is confined to tannin stained black water streams with low pH values (< 5.5) in small forested

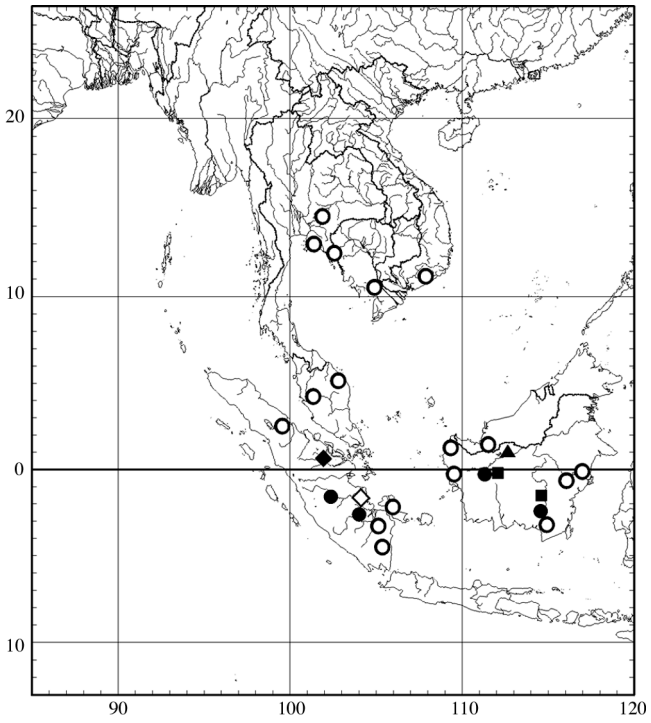

Figure 14. - Geographic distribution of the different species belonging to the Asian arowanas. Black symbols: Locations sampled in the present study. Blank symbols: Data from the literature. ▲: *S. legendrei* sp. nov.; ◆, ◇: *S. aureus* sp. nov.; ■: *S. macrocephalus* sp. nov.; ●, ○: *S. formosus*.

lakes bordering the Sentarum Lake in the upper part of the Kapuas River (West Borneo, Indonesia) (Fig. 14).

Etymology and common names

Legendrei: In honour of Marc Legendre, senior researcher in Physiology and Reproduction of Fishes at the French Institute of Research and Development (IRD). The species is commonly known as the Super Red, Chili or Blood Indonesian Arowana.

DISCUSSION

Hybridisation

Probably due to technical and economical difficulties that could arise from the constitution and the management of a Super Red broodstock, some farmers attempted to produce first generation hybrids by crossing this variety with other colored varieties such as the green or the silver one.

The most common hybrid produced in Indonesia is the Banjar Red (Fig. 15). These fishes just look like a normal Super Red when young but colour differences become evident with age (Goh and Chua, 1999). Banjar Reds never have fully red scales and anal and caudal fins become light orange with age. Five specimens of Banjar Red from various origins (MZB 11918-11922) were studied in order to establish their genetic and morphological characteristics. The cytochrome b sequences reveal that all the analysed specimens of Banjar Red are offspring of females of Yellow Tail Silvers and males of Super Red Arowanas. Morphological comparisons made between the Banjar Reds and both parental species (namely *S. legendrei* sp. nov. and *S. macrocephalus* sp. nov.) clearly establish their intermediate characteristics for most of the diagnostic characters previously given (i.e., 24.5 < PPEL < 25.8% SL; 71.5 < PAL < 75.0% SL; 30.8 < PEPL < 33.7% SL). Hybrids between Super Reds and Greens are also produced. Their offspring displays the same coloration as the Greens. Nevertheless, the identification becomes more difficult when these specimens are

Figure 15. - The pattern of coloration of the Banjar Red hybrid. A specimen of 282 mm SL (MZB 11918) obtained from a cross between a female of *S. macrocephalus* sp. nov. and a male of *S. legendrei* sp. nov.

exposed to hormones treatment to obtain a red coloration and at this stage only a detailed morphological examination is helpful.

Taxonomic status for the Cross Back Golden

This variety of Asian arowana also called the Blue Malayan bonytongue is known from the Pahang State and Bukit Merah Lake (Malay Peninsula, Malaysia). It was not possible to get specimens of this coloured variety but according to its body shape and caudal coloration (similar to that observed on *S. aureus*), we suspect that both varieties are closely related. Awaiting further taxonomic studies on the Malayan bonytongue, we propose for the moment to maintain it in *S. formosus*.

Conservation of wild populations

Scleropages formosus is considered as an endangered species, threatened with extinction and thus belongs on the IUCN Red List Species (EN A1cd+2cd; Hilton-Taylor, 2000). The present demonstration of the existence of three new species within *S. formosus* together with ecological data clearly emphasize the need for a reconsideration of its conservation status. The Super Reds and the Golden Reds which were previously considered as colour varieties of *S. formosus*, represent two distinct species (respectively *S. legendrei* and *S. aureus*) with cryptic geographic distributions highly dependant on the nature and quality of the habitat (i.e., small lakes of tannin stained black waters with low pH values). From our field studies in 2000 in the area of the Sentarum Lake (West Borneo), it appears that *S. legendrei* sp. nov. is probably extirpated from the southern area of the Lake and is only known to exist in its more protected eastern range consisting of small forested lakes (each less than 1 km^2). Information from fishermen, officials from local Department of Fisheries and farmers, indicate that the rapid decline of Super Red populations is a consequence of logging activities with water quality modifications (rise of pH and turbidity) rather than of overfishing. They argue that the disappearing of the Super Reds in the southern part of the lake occurred about ten years ago which coincided with intensive forest logging. The eastern part of the Sentarum Lake belongs to a more protected area where logging activities are theoretically forbidden, but we observed that the regulations was broken in this area leading to potential risks of deep ecological modifications in a near future. We therefore consider that *S. legendrei* sp. nov. must be rapidly considered as a critically endangered species following the IUCN classification. The same concerns must be voiced for *S. aureus* sp. nov. By opposition *S. formosus* and *S. macrocephalus* sp. nov. could be maintained in the endangered category as their habitat seems less directly vulnerable.

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REFERENCES

BANARESCU P., 1990. - Distribution and dispersal of freshwater fishes. *In:* Zoogeography of Freshwaters. General Distribution and dispersal of Freshwater Animals. Vol. 1. pp. 48-95. Wiesbaden, Germany: Aula-Verlag.

BOOKSTEIN F.L., CHERNOFF B., ELDER R., HUMPHRIES J., SMITH G. & R. STRAUSS, 1985. - Morphometrics in evolutionary biology. The geometry of size and shape change, with examples from fishes. *Acad. Nat. Sci. Philad.,* Spec. publ., 15, 277 p.

BRIOLAY J., GALTIER N., BRITO R.M. & Y. BOUVET, 1998. - Molecular phylogeny of Cyprinidae inferred from cytochrome b DNA sequences. *Mol. Phyl. Evol.*, 9: 100-108.

CABOT E.L. & A.T. BECKENBACH, 1989. - Simultaneous editing of multiple nucleic acid and protein sequences with ESEE. *Cabios*, 5: 233-234.

DOYLE J.J. & J.L. DOYLE, 1987. - A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull.*, 19: 11-15.

ESCHMEYER W.N., FERRARIS C.J. Jr., HOANG M.D. & D.J. LONG, 1998. - Part I. Species of Fishes. *In:* Catalog of Fishes, Vol. 2: Species of Fishes (M-Z) (W.N. Eschmeyer, ed.), pp. 959-1820. San Francisco: California Academy of Science.

FELSENSTEIN J., 1993. - PHYLIP (Phylogeny Inference Package) Version 3.5c. Seattle: Univ. Washington.

GOH W. & J. CHUA, 1999. - The Asian Arowana. Dragon Fish Industry, Singapore.

GREENWOOD P.H. & M.V.H. WILSON, 1998. - Bonytongues and their allies. *In:* Encyclopedia of Fishes (Paxton J.R. & W.N. Eschmeyer, eds), pp. 80-84. San Diego: Academic Press.

HILTON E.J., 2003. - Comparative osteology and phylogenetic systematics of fossil and living bony-tongue fishes (Actinopterygii, Teleostei, Osteoglossomorpha). *Zool. J. Linn. Soc., Lond.*, 137: 1-100.

HILTON-TAYLOR C., 2000. - 2000 IUCN red list of threatened species. IUCN, Gland, Switzerland and Cambridge, UK. xviii + 61 p. (with 1 CD-ROM).

INOUE J.G., MIYA M., TSUKAMOTO K. & M. NISHIDA, 2001. - A mitogenomic perspective on the basal teleostean phylogeny: Resolving higher-level relationships with longer DNA sequences. *Mol. Phyl. Evol.*, 20(2): 275-285.

KOTTELAT M., WHITTEN A.J., KARTIKASARI S.N. & S. WIRJOATMODJO, 1993. - Freshwater Fishes of Western Indonesia and Sulawesi. 259 p. Hong Kong: Periplus Editions.

KUMAZAWA Y. & M. NISHIDA, 2000. - Molecular phylogenetic analysis of vertebrate radiations. *In:* IIAS International Symposium on Biodiversity, Dec. 3-5, 1998 (IIAS Reports No. 1999-003) (K. Iwatsuki, ed.), pp. 137-143. Kyoto, Japan: International Institute for Advanced Studies.

MÜLLER S. & H. SCHLEGEL, 1844. - Beschrijving van een' nieuwen Zoetwater-visch van Borneo, *Osteoglossum formosum. Verh. Natuur. Gesch. Leiden*, 1-7.

NELSON J.S., 1994. - Fishes of the World. 3rd edition. 600 p. New York: J. Wiley & sons.

ROBERTS T.R., 1989. - The Freshwater Fishes of Western Borneo (Kalimantan Barat, Indonesia). *Mem. Calif. Acad. Sci.*, 14: 1-210.

SAITOU N. & M. NEI, 1987. - The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.*, 4: 406-525.

SANGER F., NICKLEN S. & A.R. COULSON, 1977. - DNA Sequencing with chain terminating inhibitors. *Proc. Nat. Acad. Sci.*, 74: 5436-5467.

VORIS H.K., 2000. - Maps of Pleistocene sea levels in Southeast Asia: Shorelines, river systems and time durations. *J. Biogeogr.*, 27: 1153-1167.

WEBER M. & L.F. de BEAUFORT, 1913. - The Fishes of the Indo-Australian. Arch. II. Malacopterygii, Myctophoidea, Ostariophysi: I. Siluroidea, 404 p. Leiden: E. Brill, Ltd.

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